PORTABLE MICROFLUIDIC BIOLOGICAL AGENT DETECTION SYSTEM

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ABSTRACT

A portable system has been developed for rapid detection of biological warfare agents. The system is based on coupling highly specific immunoassay technologies with ultra-sensitive electrochemical detection. The detection system utilizes low-cost, disposable microfluidic cartridges and is capable of detecting as low as 700 cells/ml of pathogenic bacteria in 60 minutes. The system is optimized for detection of biological agents in liquid samples and in air by integration with portable aerosol collectors. The developed system can be applied to protection of forward deployed critical infrastructure and troops.

1. INTRODUCTION

Rapid detection and quantification of biological entities is a pressing need, now more than at any time in history. The recent casualties from anthrax developed by contamination through the postal system are the first documented domestic deaths from biological warfare. The threat of further widespread attacks with biological agents continues to build. The use of cell cultures is insufficient because of the several-day waiting period and the size of workforce required for analysis of periodic samples from all locations of possible attack. A technology is urgently needed that can identify the presence of a biological agent before it is spread over a large geographical area. Furthermore, this technology must operate reliably without a substantial need for human intervention.

In recent years, a number of techniques have been under development making detection and identification of biothreat agents faster and more sensitive. Two significant technologies that impacted rapid detection are nucleic acid-based and antibody-based technologies (Ivnitski et al, 1999). The majority of nucleic acid based approaches involve some form of target amplification (for example through the use of PCR) followed by the use of a reporter system (such as enzymatic, fluorescent or chemiluminescent labels). Even though these nucleic acid based approaches are highly sensitive and specific, the analysis procedure is usually very complex requiring highly specialized instrumentation and a high degree of automation. In addition, most nucleic acid based approaches require reagents with special storage conditions. Size, robustness, automation, and ruggedness of these systems has not reached a stage allowing their deployment in field conditions.

On the other hand, immunological detection techniques that utilize antibodies are the only technology that has been successfully employed under field conditions. In addition, antibodies have been successful in the detection of bacterial cells, spores, viruses and toxins alike in a variety of complex media. In particular enzyme-linked immunoassays have been established as a standard technique for the detection of a number of biological substances in both clinical and military applications. Despite the robustness of this technique, its sensitivity for some applications, such as early detection of biothreat agents, has not been satisfactory, requiring long incubation periods.

In this manuscript, we report on the development of portable, semi-automated immunoassay system that is capable of detecting low concentrations of biological agents. The sensitivity of the system has been greatly enhanced by the use of a dual enzyme amplification system.

2. PRINCIPLE OF DETECTION

The detection of pathogens and/or proteins is based on an immuno-electrochemical principle. The sample to be analyzed is mixed with enzyme-labeled antibodies towards the bioagents of interest. After antibody binding, the labeled sample passes through a capture surface that selectively binds the pathogen/protein target of interest while allowing unbound targets and antibody-enzyme conjugates to be removed. The amount of bound enzyme is directly proportional to the number of captured pathogens/proteins (Abdel-Hamid et al, 1999). This process is illustrated in Figure 1.

Quantification of bound enzyme is performed using a miniature electrochemical sensor. The amperometric electrochemical sensor is based on the detection of electroactive substances that are formed as a result of the antibody-linked enzyme-catalyzed reactions. Antibody-conjugated horseradish peroxidase was used as the enzymatic label and hydrogen peroxide and iodide ions

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used as substrates. Horseradish peroxidase catalyzes the oxidation of the iodide ions by hydrogen peroxide to give Iodine. This iodine is then electrochemically reduced at the working electrode surface with an uptake of two electrons. The flux of electrons out of the working electrode is measured in the form of a current signal. The magnitude of the current signal is proportional to the number of biological agents that have been captured and labeled.

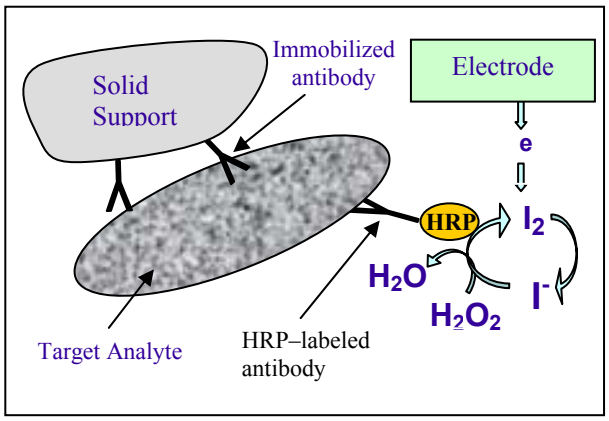

Fig. 1 PRINCIPLE of operation of the electrochemical immunoassay system.

3. MICROFLUIDIC IMPLEMENTATION

In order to miniaturize the system and to achieve a low cost disposable, the above steps have been implemented in a microfluidic format shown in Fig. 2. This microfluidic cartridge measures 4 by 5 inches and houses the reagent reservoirs, antibody labeling and concentration chambers as well as a disposable screen-printed electrochemical sensor.

Fig. 2 MICROFLUIDIC disposable cartridge

The microfluidic cartridge is fabricated using laser-cut Mylar layers. Multiple layers of plastic are laminated together to build a three dimensional cartridge. Each layer contains a unique pattern of channels and reservoirs, allowing creation of complex three-dimensional fluid paths (Weigl et al, 2001). The individual layers can be manufactured with adhesive on one or both sides, allowing lamination of as many layers as desired. This method of fabrication was selected due to many advantages, including very low cost, flexibility, speed, and accessibility. Care was taken to ensure that all designed cartridges could be replicated using injection molding. From the long-term perspective of sales of thousands of microfluidic cartridges, injection molding is a very cost-effective means of large-scale production.

4. PORTABLE SYSTEM DESIGN

Ease-of-use, automation and ruggedness are key elements for field deployment of any detection system. A bench-top semi-automated prototype of the detection system was developed and is shown in Fig. 3.

Fig. 3 PROTOTYPE of the automated detection system

The prototype is built around a fluidics system (pumps, valves and interconnects) for moving the reagents and fluids in the correct sequence for conducting the analysis. An electronics subsystem performs automation and control of individual component operation as well as data acquisition and processing. When the microfluidic cartridge is loaded into the system, it is automatically pierced in several locations, giving fluidic access to each reservoir.

5. SYSTEM PERFORMANCE

The bench-top unit performance has been demonstrated in the analysis of pathogenic bacteria and proteins. Fig. 4 demonstrates the performance of the developed system with respect to the detection of low concentrations of *E. coli* cells. It can be seen that the system can detect less than 700 cells/ml with an overall assay time of 60 minutes. Further reduction of the overall assay time is possible with the utilization of improved amplification systems.

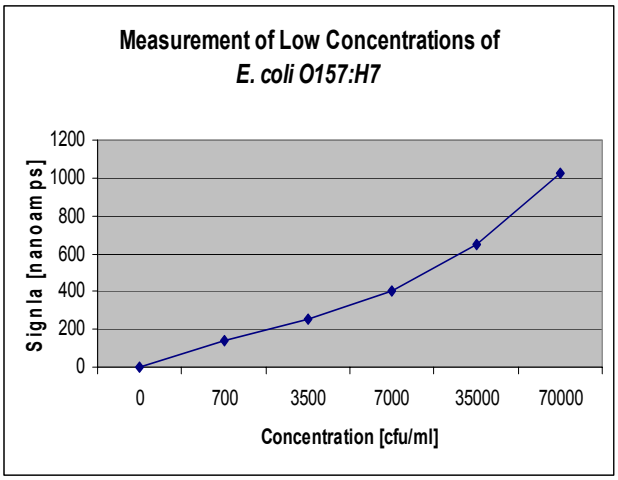

Fig. 4 E. COLI detection using the developed system

The detection of low concentrations of proteins, and hence toxins, was also demonstrated. Fig. 5 demonstrates the detection of low concentrations of Immunoglobulin G (use as a representative protein). It can be seen that the system can detect less than picoMolar concentrations of the protein. The overall assay time for detection of proteins is 40 minutes.

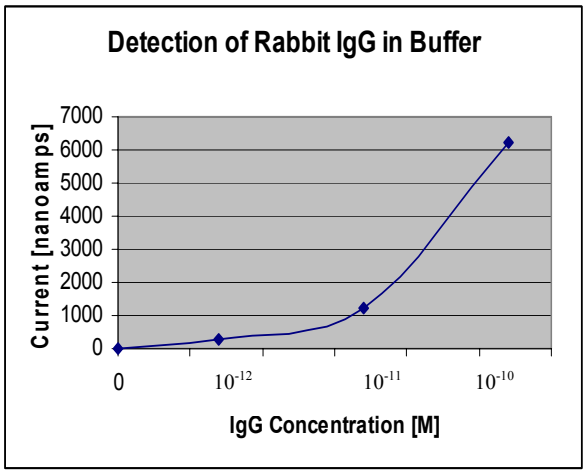

Fig. 5 IgG detection using the developed system

CONCLUSIONS

A portable microfluidic biological agent detection system has been developed. The detection limit of the system can be as low as 700 cells/ml with an assay time of 60 minutes for pathogenic cells and as low as 0.1 nanograms/ml with an assay time of 40 minutes for proteins and toxins. The detection system can also be applied to detection monitoring of airborne pathogens by integrating with MesoSystems BioCapture series of high-capture efficiency aerosol collectors. Current efforts are focused on achieving full-automation of the system to allow autonomous and remote operation. Furthermore, work on achieving a robust and stable disposable cartridge that can be stored and used under field conditions is under way. The developed system will have applications in the protection of forward deployed troops and facilities as well as in homeland security applications.

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